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# Conservation Analysis of Functional Important Residues of the Oxygen Evolving Mechanism Located in the D1 Subunit of Photosystem II

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Key residues of the oxygen evolving center of Photosystem II are examined by a conservation analysis using a previously constructed profile Hidden Markov Model. The analysis revealed for some of the crucial residues a unexpected flexibility of the aminoacid character.

## 1 Introduction

In the evolution of life on earth, oxygen producing photosynthesis is of central importance. The oxygen evolving center is part of Photosystem II in plant chloroplasts and is located near to the luminal site in the reaction center formed by the subunits D1 and D2 of Photosystem II. Water binds to the manganese cluster of the reaction center. During the water splitting reaction, electron and protons are abstracted from water involving the nearby Tyrosine $Y_Z$ (Tyr161 of the D1 subunit). After four oxidation steps, molecular oxygen is released. From $Y_Z$, protons are transferred via several protein residues to the lumen and the electrons are used to restore the oxidized special pair. It is known that in addition to $Y_Z$ and the manganese cluster, a calcium ion and a chloride ion are needed for the oxygen formation, but the detailed mechanism of oxygen formation is still under debate. Even in the available crystal structures of Photosystem II with reasonable resolution, the exact organization of the manganese cluster is not clear.[1,2] However, several residues in the D1 subunit were suggested to coordinate the oxygen evolving center or to influence the oxygen formation due to interactions with $Y_Z$[3]. Namely Asp170, His332, Glu333, His337, His342 and the C-terminal of Ala344 are likely to coordinate manganese ions; Glu189 is suggested to coordinate the calcium ion and to be part of proton transfer pathway from $Y_Z$;[3] His190 abstracts a proton from $Y_Z$.[4]

In the here presented work, the conservation of functionally important residues is analyzed by sequence alignment using a previously constructed profile Hidden Markov Model.[5] Our analysis indicates that some of the functional important residues are not as strictly conserved as one might expect.

## 2 Material and Methods

Using a previously constructed profile Hidden Markov Model of the D1 subunit of Photosystem II and its ancestor subunit of bacterial reaction centers,[5] 226 D1 sequences were aligned using the HMMER software.[6] The so-constructed alignment was used for the conservation analysis.

| Function | Residue | C (%) | ex | (%) | ex | (%) |
|---|---|---|---|---|---|---|
| Manganese coordination | Asp170 | 98.7 | **Asn** | **0.9** | *His* | *0.4* |
| | His332 | 100.0 | | | | |
| | Glu333 | 100.0 | | | | |
| | His337 | 100.0 | | | | |
| | Asp342 | 100.0 | | | | |
| | Ala344 | 100.0 | | | | |
| $Y_Z$ function | Tyr161 | 100.0 | | | | |
| | His190 | 100.0 | | | | |
| Calcium binding | Glu189 | 95.1 | **Asp** | **3.5** | *Lys* | *1.3* |

Table 1. Conservation analysis of residues of the D1 subunit suggested to be involved in oxygen formation. For each residue the conservation (C) and the exchange (ex) is given in %. Bold characters mark aminoacid exchanges, for which mutational studies in the cyanobacterium *Syncheocystis* sp. PCC 6803 that this mutant does not grow photoautotrophicallly. Italic character mark, aminoacid exchanges for which mutational studies in *Syncheocystis* sp. PCC 6803 showed, that the mutant still grows photoautotrophically.[3,7,8]

## 3 Results and Discussions

For all examined residues (see Table 1), a high conservation is observed, which is not surprising, since the sequences of the D1 subunits show an average sequence identity of about 85 %. Experimental evidence exists, that Asp170 is part of the high-affinity binding site for the first manganese ion which is first photo-oxidized during the light-driven assembly of the cluster.[9] Whether Asp170 also ligates the manganese ion, which is first photo-oxidized during the oxygen evolving reaction is still a matter of debate.[8–10] Because of its importance one would expect that especially Asp170 should be strictly conserved. Moreover, mutational studies showed that an exchange of Asp170 abolished oxygen evolution in most cases.[8] Nevertheless our study indicates that Asp170 is not strictly conserved in contrast to the other proposed manganese coordinating coordinating residues. We also observe an Asn and an His at the position 170. Asp170→His mutants are photoautothrophic, but in Asp170→Asn mutants oxygen evolution is nearly abolished.[8] It is possible that the exchange of Asp→Asn is only an artifact of the protein sequencing technique. But it might also be that although Asp170 is very important for the oxygen evolution, the protein can restore the function without this residue, maybe by second site mutations.

Tyrosine $Y_Z$ (Tyr161) is crucial for the function of the Photosystem II reaction center, since through its radical state, it transports the electrons from the water (bound at the manganese cluster) back to the special pair and accepts also the protons during water splitting. Since it is assumed, that $Y_Z$ is initially protonated, a base is needed near to $Y_Z$ to abstract the proton of $Y_Z$. His190 has been suggested to be this base.[4] Both His190 and Tyr161 are strictly conserved in our analysis, which shows their crucial importance in the reaction mechanisms. Glu189 was proposed to accept a proton from His190 and thus to be part of a proton transport system. Moreover, it is suggested to coordinate the calcium ion.[2] Our study shows that Glu189 is not strictly conserved. The mutation Glu189→Asp in the cyanobacterium *Syncheocystis* sp. PCC 6803 led to organisms that could not grow photoautothrophically. However, we observe an Asp at the position of Glu189 in some of

the D1 sequences. Thus, it might be, that either another residue can take over the function of Glu189 or that organisms with Asp189 differ in their proton transfer mechanism.

## 4 Concluding Remarks

The functionally crucial residues such as Tyr161 and His160, as well as several suggested manganese coordinating residues (His332, Glu333, His337, Asp342 and Ala344) are strictly conserved. Surprisingly the residues Glu189 and Asp170, which are also thought to be very important for the function of $Y_Z$ and the manganese cluster, are not conserved in the same extent. Moreover, they are also exchanged to aminoacids for which it is known that these mutations prevent oxygen production in the cyanobacterium *Syncheocystis*. It might be that although Asp170 and Glu189 are very important for the oxygen evolution that the protein function can be restored without these residues by second site mutations.

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